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LIFE CYCLE IMPACTS OF BIOPROCESSES

Enzymes for pharmaceutical applications—a cradle-to-gate life cycle assessment

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Abstract
Background, aim, and scope During the last decade, the interest in estimating environmental life cycle impacts of bioprocesses has markedly risen. To adequately quantify these impacts, accurate life cycle inventories of materials, such as agricultural substrates and enzymes, are required. The goals of this life cycle assessment were (1) to estimate the life cycle inventories (LCIs) and impacts of three supported enzymes produced in-house for pharmaceutical applications (A, B, and C) and (2) to determine the suitability of applying modular life cycle inventory estimation techniques to enzymes when individual enzyme LCIs are not readily available. The scope of this LCA was cradle to gate, covering the production and purification of the enzymes, energy generation, raw material production, waste treatment, and transportation of the raw materials.
Materials and methods Three immobilized enzymes (A, B, and C) produced industrially for application in pharmaceutical products were studied. Enzyme production information was obtained from internal process descriptions. LCI information was obtained from GlaxoSmithKline's in-house LCA database FLASC™, from LCA commercial databases, and literature. The LCI for the enzyme support was estimated using its material flows. Mass allocations were applied to multi-output processes in the upstream processes. The life cycle impacts considered were nonrenewable energy consumption, global warming, acidification, eutrophication, and photochemical smog formation.
Results and discussion Life cycle impacts of the immobilized enzymes A, B, and C were estimated. For instance, nonrenewable energy use is between 117 to 207 MJ/kg of immobilized enzyme and the global warming potential ranges from 16 to 25 kg CO_2 eq/kg immobilized enzyme. Contributions of different subprocesses were also estimated. For example, support production accounts for about 31% to 67% of the energy consumption and soybean protein and yeast extract account for about 64% to 72% of the total photochemical smog formation. Uncertainty and sensitivity analysis were performed using Monte Carlo simulation and showed that a standard deviation of the environmental impact is less than 7% of the mean in all the environmental impacts considered. "What if" analysis shows that using biobased glycerin instead of petroleum-based glycerin could reduce global warming impacts between 11% and 44%.
Conclusions, recommendations, and perspectives The production of immobilized enzyme is, in general, energy intensive. Enzyme A has larger environmental impacts than the other enzymes evaluated because of larger energy intensity and lower enzyme production yield. The media preparation inputs (soybean protein, yeast extract) and immobilization subprocesses are the two major contributors to acidification, eutrophication, and photochemical smog formation. Immobilization is the major contributor for global warming potential. "What if" analysis estimated changes on life cycle impacts for biobased vs. synthetic substrates. The results of this LCA are, in general, comparable with results previously reported in the literature

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(Nielsen et al., Int J Life Cycle Assess 12(6):432–438, 2007). Therefore, using this technique to estimate LCI of enzymes appears to be suitable for future life cycle assessments of biocatalyzed processes. The results of this study will be integrated into GlaxoSmithKline's FLASC™ to improve the accuracy of life cycle assessment for biocatalyzed processes and enzymes produced in-house.

Keywords Biocatalysis · Bioprocesses · Cradle-to-gate · Enzymes · LCA · Life cycle assessment · Life cycle inventories · Pharmaceuticals

1 Background, aim, and scope

During the last 10 years or so, there has been a broader interest in estimating the life cycle impacts of bioprocesses and how these compare with the more traditional chemical processes. The intuitive benefits of biocatalyzed reactions has resulted in the consideration of a significant number of bioprocesses and biocatalyzed plus synthetic strategies for potential pharmaceutical manufacture (Straathof et al. 2002; Schoemaker et al. 2003). Although the application of biocatalysis in pharmaceutical applications at a large scale is still restricted due to the limited number of enzymes commercially available, the use of regioselective and stereoselective enzymes in pharmaceutical applications is promising. Furthermore, there are potential cost and environmental improvements that can be associated with the use of biocatalysis (Sheldon 1994; Bull et al. 1999; Rozell, 1999).

Although scientific intuition indicates that bioprocesses could potentially bring environmental and cost improvements (Sheldon 1994; Bull et al. 1999; Rozell 1999), this intuition must be quantitatively substantiated through a scientifically rigorous comparison. The experience of the authors when quantitatively comparing bio- and chemical processes is that bioprocesses do not always exhibit a marked superiority from the life cycle environmental viewpoint (Jödicke et al. 1999; Henderson and Jiménez-González 2007). The relative environmental life cycle profiles depend not only on the biocatalytic reactions in the synthesis but more importantly on the downstream processes for the purification and isolation of the desired product. This is particularly important in the case of pharmaceutical products, where the separation train that follows the bioreactions can bring significant life cycle burdens that can negate some of the advantages of the enhanced efficiency and improved regio- and stereoselectivity of enzymes. It is therefore important to not only focus on the biosynthesis performance itself but also to account for the wider process and life cycle implications. A life cycle assessment of bioprocesses can also serve to identify "hot spots" that need to be revisited in development (e.g., eliminate or optimize extractions with organic solvents) to render an improved bioprocess with a smaller environmental footprint.

There have already been several attempts to quantitatively estimate the life cycle environmental impact of bioprocesses compared to chemical processes (Bayer 2004; Henderson et al. 2008). These studies have faced the challenge of not having sufficient life cycle inventory (LCI) information available to adequately estimate environmental burdens and compare the environmental impacts and footprint of bioenzymatic process with synthetic chemical routes, thus resulting in a series of assumptions and estimations.

Enzymes play a pivotal role in biocatalyzed synthesis, and having accurate life cycle inventory information for enzyme production (cradle-to-gate) could enable better comparisons between these types of processes. Cradle-to-gate life cycle impacts of commercially available enzymes have been reported in the literature (Nielsen et al. 2007), but additional data for enzymes are required for the different types of commercially available enzymes. Furthermore, many enzymes are produced internally and the production processes are oftentimes confidential. Being able to estimate the environmental life cycle footprint of these enzymes using standard chemical engineering and life cycle techniques might make it possible to effectively and efficiently estimate the life cycle of bioprocesses using a modular approach.

With that in mind, the goal of this research is twofold: first, to estimate the life cycle impact of three supported enzymes produced in-house in GlaxoSmithKline (GSK; A, B, and C) and second, to determine the suitability of applying modular life cycle inventory estimation techniques, so these estimation techniques can be used to evaluate the life cycle profiles of biocatalyzed processes when enzyme LCIs are not readily available.

To achieve this aim, the case study was cradle to gate in scope, covering the production and purification of the enzyme, energy generation for enzyme production, raw material production, enzyme process waste treatment, and transportation of the raw materials. Since the life cycle inventory and impact estimations are intended to be applied in wider life cycle assessments of bioprocesses, the use phase of the enzyme in biocatalytic processes is outside the scope of this assessment.

2 Materials and methods

This case study involves the cradle-to-gate life cycle assessment of three supported, or immobilized, enzymes that have been produced industrially in-house for application in pharmaceutical products by GlaxoSmithKline.

These enzymes are an aldolase, a carbamoylase, and a hydantoinase and have been labeled A, B, and C, respectively. Enzyme production information, mass, and energy flows were obtained directly from internal GSK standard operating procedures and process descriptions. For this case study, the functional unit is 1 kg of immobilized enzyme. The system boundary includes most processes involved in the immobilized enzyme production system and is illustrated in the simplified process flow diagram shown in Fig. 1. The following enzyme production processes and subprocesses for the enzymes analyzed and their boundaries are included:

- Media preparation includes the upstream processes for substrate production (including transportation) and energy consumption in substrate mixing.
- Fermentation includes energy consumption in agitation and heating during enzyme production and emissions during fermentation. Sanitization includes steam consumption in sanitization.
- Separation includes water consumption and energy consumption in separating cells from the fermentation broth.
- Cell disruption includes energy consumption and chemicals used.
- Immobilization includes the enzyme support sepabead (poly(glycidyl methacrylate-co-ethylene dimethacrylate)), chemicals, and energy consumption in immobilizing enzyme in the sepabead.
- Waste management includes a wastewater treatment facility and assumes biosolids are discharged into the sea.

Note that enzyme used in inoculation is not included in the system boundary due to lack of data and its small quantity.

LCI information was obtained from the GSK in-house LCI database FLASC™ (Curzons et al. 2007), from commercial LCI databases such as DEAM™ and Ecoinvent (Ecobilan 2009 and EMPA 2009), and the literature (Kim and Dale 2005). The LCIs in FLASC™ had been developed for over a decade using a modular approach that applies chemical engineering principles to the estimation of life cycle inventories when published LCI data are not available (Jiménez-González et al. 2000; Jiménez-González et al. 2001; Curzons et al. 2007). Steam used during the steps of enzyme production assumes a fuel mixture of 50% natural gas and 50% fuel oil, which is representative of the industrial conditions and with environmental burdens previously published (Jiménez-González and Overcash 2000). LCIs for agricultural processes involved in biobased substrates (e.g., soybean protein, yeast extract, etc.) include the environmental burdens associated with the direct land use change, measured by changes in soil organic carbon levels (Kim and Dale 2005). Using biobased substrates is

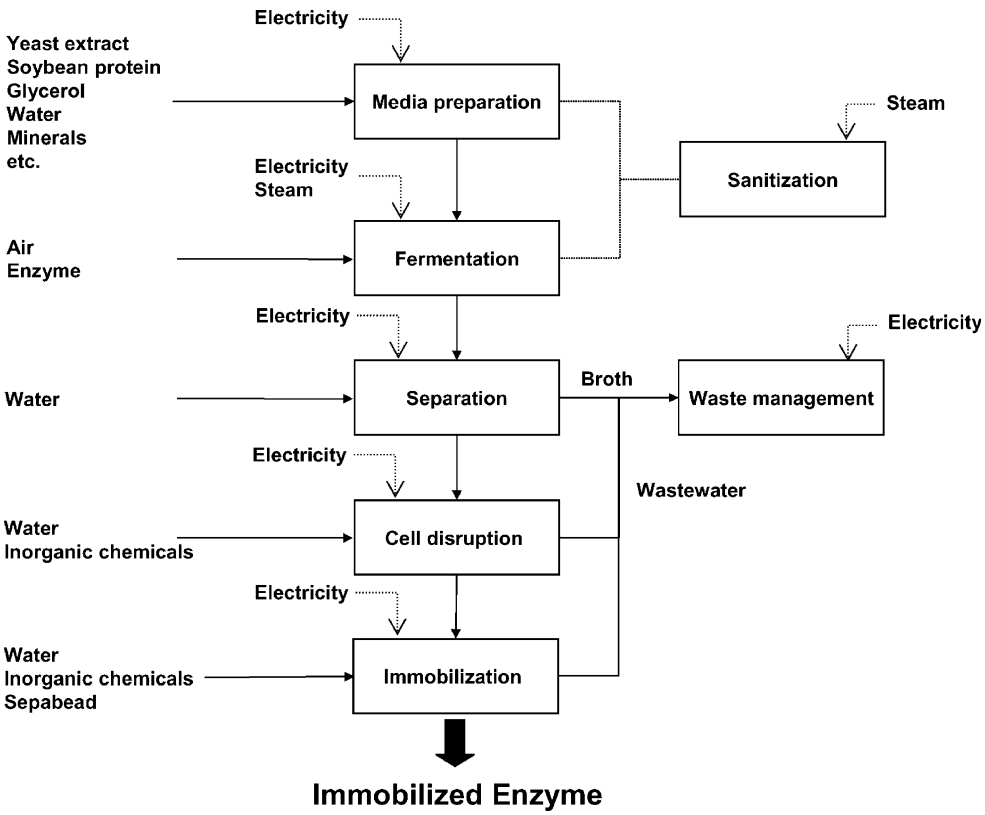

Fig. 1 Generic enzyme production process

unlikely to convert undisturbed natural ecosystems to croplands because of the small quantities involved. Hence, the land use change associated with conversion of undisturbed natural ecosystems to croplands (indirect land use change) is not included in the analysis. In addition, it is highly debatable whether or not indirect land use change can appropriately be dealt with by LCA (Kim et al. 2009). Soil organic carbon levels are simulated by the DAYCENT model (Del Grosso et al. 2001). The LCI for sepabead production was estimated with its material flows: glycidyl methacrylate, ethylene dimethacrylate, epichlorohydrin, methacrylic acid, ethylene glycol, methacrolein, and so forth (Hosokawa et al. 1990; Kuroda et al. 1993; Abe et al. 1995). Process energy use for these chemicals is estimated from mean energy consumption for organic chemical manufacture (Kim and Overcash 2003). Mass allocations are applied to multi-output processes in the upstream processes (i.e., soybean protein and yeast extract).

The environmental impacts considered in this study are nonrenewable energy consumption, global warming, acidification, eutrophication, and photochemical smog formation. The 100-year time horizon global warming potentials (Intergovernmental Panel on Climate Change 2001) are used to estimate greenhouse gas emissions. Acidification, eutrophication, and photochemical smog are estimated by the CML characterization factors (Guinée 2002). Other environmental impacts (e.g., ecotoxicity, human toxicity, etc.) are not included in the analysis due to the lack of information. Nonetheless, both human and ecotoxicity are very important impacts that the authors wish to address in future research work, as more information becomes available and as the life cycle impact factors for toxicity become standardized worldwide (Rosenbaum et al. 2008).

3 Results and discussion

3.1 Environmental impacts

The environmental impacts associated with the supported enzyme production systems A, B, and C are presented in Fig. 2. Global warming impacts of the immobilized enzyme production systems range from 16 to 25 kg CO_2 eq/kg of immobilized enzyme. Enzyme A produces more environmental impacts than other enzymes because of (1) energy intensity and (2) lower enzyme yield. Most subprocesses in the enzyme A system consume more energy (i.e., electricity and steam) than other enzymes, especially the sanitization subprocess. The immobilization subprocess, particularly sepabead production, is the primary greenhouse gas emission source for the immobilized enzyme (51 to 83%), followed by the sanitization subprocess. The sanitization subprocess contributes about 9% to 19% of the overall global warming impacts, and the major greenhouse gas (GHG) source in the sanitization subprocess is steam generation. GHG emissions associated with the waste management subprocess account for about 2% to 15% of the overall global warming impacts of the immobilized enzyme production systems. CO_2 produced from wastewater treatment is the primary GHG source in the waste management subprocess. This CO_2 is produced from the degradation of the organic carbon contained in the influents to the facility, which are composed of wastewater, fermentation broth, and disrupted cell residues. There are two main sources of organic carbon in the influents: that which is biologically derived and that which is petroleum based. Sources for bio-origin carbon in the fermentation broth and disrupted cell residues are biobased substrates (e.g., soybean protein, yeast extract, etc.). CO_2 released from bio-origin carbon is not regarded as a greenhouse gas emission under the assumption that the bio-origin carbon has been sequestered from the atmosphere as part of the carbon cycle through photosynthesis. Therefore, only petroleum-based CO_2 emissions are accounted for as greenhouse gases. In this case study, glycerin is the only petroleum-based substrate considered in the media preparation (the potential of using biobased glycerine is discussed later in this paper). About 0.3–0.8 kg of CO_2/kg of immobilized enzyme is released during fermentation. Atkinson and Mavituna (1991) show that about 32% to 48% of carbon in the substrate is converted into CO_2 during fermentation. The mean fraction (38%) is used in estimating the quantity of CO_2 released during fermentation. Petroleum-based CO_2 accounts for about 13% to 53% of the total CO_2 released during fermentation. Note that the fermentation process in Nielsen and his colleagues' study (2007), which is one of the primary GHG sources, is equivalent to a subprocess group including media preparation, fermentation, and sanitization in this study. Results from this study also show that this subprocess group is one of the primary GHG sources.

Immobilized enzyme production is energy intensive, consuming about 117 to 207 MJ of nonrenewable energy per kilogram of immobilized enzyme. Energy intensive subprocesses are immobilization, sanitization, media preparation, and waste management. Most nonrenewable energy in the immobilized enzyme production systems is consumed in producing sepabead, accounting for about 31% to 67% of the total energy consumption, followed by steam generation in sanitization (19 to 36%). The immobilization subprocess is the primary acidification contributor, particularly due to energy consumption in sepabead production. Sulfur oxides are the primary acidification pollutants. The media preparation subprocess is the primary eutrophication source in enzyme A, while the immobilization subprocess is the primary eutrophication source in enzymes B and C.

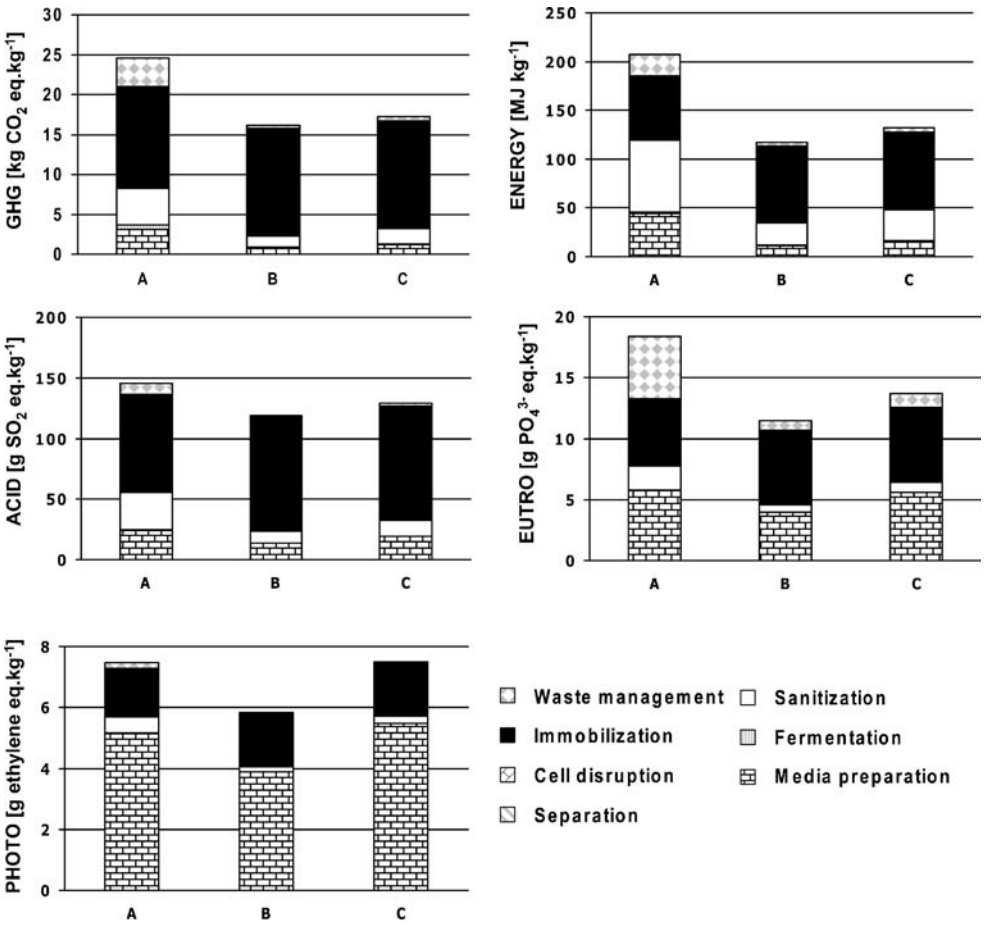

Fig. 2 Selected environmental impacts associated with the immobilized enzyme production systems *A*, *B*, and *C* [*GHG* global warming, *ACID* air acidification, *EUTRO* eutrophication, *PHOTO* photochemical smog formation, *ENERGY* nonrenewable energy consumption]

Soybean protein and yeast extract in the media preparation subprocess accounts for about 24% of the total eutrophication of enzyme A because of nutrient losses (primarily nitrogen compounds) during crop cultivation. The media preparation subprocess is the primary source of photochemical smog formation. Soybean protein and yeast extract together account for about 64% to 72% of the total photochemical smog formation of the immobilized enzyme. Hexane, ethanol, and nitrogen oxides are the primary pollutants of photochemical smog formation in soybean protein, yeast extraction, and sepabead, respectively. Hexane is used as a solvent to extract soybean oil in soybean protein production. Ethanol is a coproduct of yeast extract.

3.2 Uncertainty and sensitivity analysis

Uncertainties associated with the input parameters (e.g., electricity, steam, substrate, etc.) were determined by Monte Carlo simulation, which can also identify the most important environmentally sensitive areas. It was assumed that the inputs have a lognormal distribution function with a standard deviation equal to 10% of the given value. Monte Carlo simulations show that a standard deviation of the environmental impact is less than 7% of the mean in all the environmental impacts considered in this study. From these results, glycerine, soybean protein, and yeast extract in the media preparation, sepabead in immobilization, steam in sanitization, and electricity consumption in waste management are the most environmentally sensitive input parameters. The percentages of the environmental impacts of these input parameters are illustrated in Fig. 3.

Sepabead production is one of the most environmentally sensitive factors associated with the immobilized enzymes, and its LCI is estimated based on an assumption that 95% of the raw materials (i.e., glycidyl methacrylate and ethylene dimethacrylate) are involved in polymerization of the sepabead. Raw material production accounts for most of the environmental impacts associated with sepabead. Lowering the conversion rate of raw materials by 11% could increase the environmental impacts associated with the immobilized enzyme production systems by about 2% to 8%. The conversion rate of raw materials affects global warming and acidification more than other impacts because sepabead manufacture contributes more to these two impacts.

We assumed that about 38% of the carbon content of the substrate is converted into CO_2 during fermentation. Sensitivity analysis shows that this assumption does not significantly affect the final results. Contribution rates of

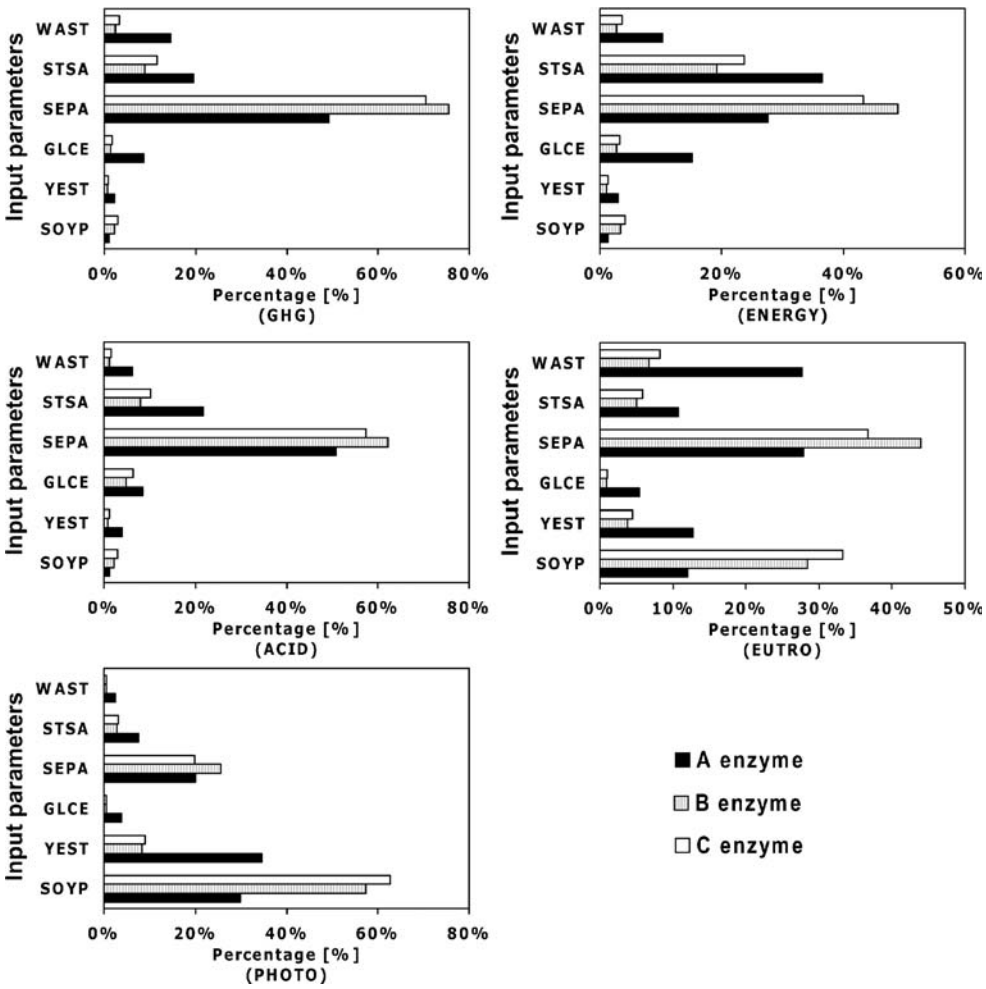

Fig. 3 Environmentally sensitive input parameters for enzymes *A*, *B*, and *C* [*SOYP* soybean protein in media preparation, *GLCE* glycerin in media preparation, *SEPA* sepabead in immobilization, *STSA* steam in sanitization, *ELWA* electricity in waste management, *WASWA* wastewater treatment facility in waste management]

the individual subprocesses to the overall impacts can be changed in the analysis. Assuming that a high fraction of the carbon content in the substrate is converted to CO_2 during fermentation, the contribution of fermentation to global warming increases, while the contribution of waste management decreases. At lower fractional conversion in fermentation, the opposite trend occurs.

3.3 Comparison with other reported enzyme LCAs

To evaluate the suitability of applying these modular life cycle estimation techniques, the LCA results were compared with enzyme LCA reported previously in the literature by Novozymes A/S. To compare the environmental impacts associated with enzymes produced by GSK to those of enzymes produced by Novozymes A/S (Nielsen et al. 2007), the immobilization subprocess is excluded because enzymes produced by Novozymes A/S are not immobilized enzymes, and the environmental impacts of the GSK enzymes are recalculated with Eco-indicator 95 that is used in Nielsen and his colleagues' study. The environmental impacts associated with enzymes produced by GSK are of the same magnitude as those reported by Novozymes. Enzymes B and C are well within the previously reported range. In the case of acidification, enzyme A generally has larger environmental impacts than enzymes B and C and the enzymes reported by Novozymes A/S. For instance, enzyme A's acidification impact is larger by up to a factor of two than those of enzymes produced by Novozymes A/S and those of enzymes B and C. This is illustrated in Fig. 4. One possible reason for higher environmental impacts in one of the enzymes produced by GSK is the lower enzyme yield exhibited for enzyme A. Nonetheless, the range of the life cycle impacts of the GSK enzymes is comparable to the life cycle impacts reported by Novozymes, as shown in Fig. 4.

3.4 "What if" analysis—use of biobased vs. synthetic glycerin

Biobased glycerin is available from biodiesel production (Sheehan et al. 1998), which is a multi-output process. There are several feasible allocation methods for assigning the burdens of biodiesel production to glycerin. We chose

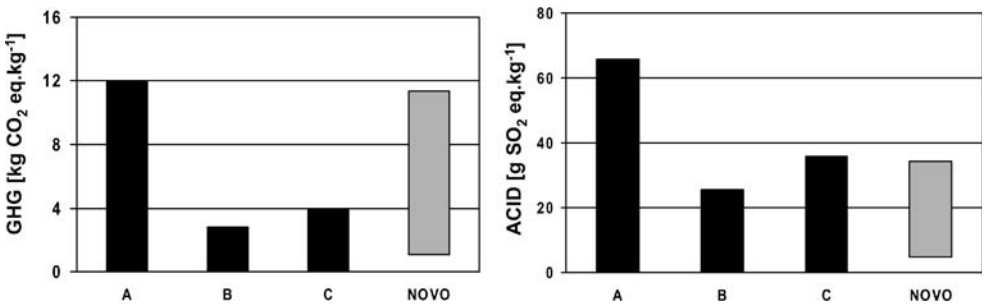

Fig. 4 Comparisons of selected environmental life cycle impacts of enzymes *A*, *B*, and *C* with previously published data [*NOVO* ranges of impacts associated with enzymes produced by Novozymes A/S (Nielsen et al. 2007)]

the mass allocation and system expansion approach to estimate the LCI of glycerin. Economic-based allocation is not included because of the recent volatility in crude oil prices. The alternative product system in the system expansion approach includes the diesel production system (including precombustion and combustion) and the combustion process of biodiesel. LCIs for diesel production can be obtained from a commercially available LCA database (DEAM™), and LCIs for the biodiesel combustion process were taken from a report by the US EPA (Office of Transportation and Air Quality 2002). The system expansion approach is done on an equal heating value basis. Greenhouse gas emissions associated with land use change (direct and indirect), measured by changes in soil organic carbon levels, are included in the analysis with the assumption that undisturbed ecosystems converted to croplands (corn–soybean rotation) consist of 62% of grassland and 38% of forest (Searchinger et al. 2008). While indirect land use change is highly controversial, we included it here for the sake of completeness. Greenhouse gas emissions associated with land use change are 178 g CO_2 eq/kg of biobased glycerin in the mass allocation method and 1,013 g CO_2 eq/kg of biobased glycerin in the system expansion approach (Kim et al. 2009). When the effects of indirect land use change are not included, greenhouse gas emissions associated with land use change (i.e., direct land use change) become credits because conservation tillage is dominant in soybean cultivation. Using biobased glycerin instead of petroleum-based glycerin affects global warming more than other environmental impacts. The environmental impacts associated with other enzymes are not greatly affected by using biobased glycerin because of the low contribution of glycerin to the total environmental impacts. Using biobased glycerin instead of petroleum-based glycerin reduces the overall global warming of enzyme A by 11% in the mass allocation method and by 44% in the system expansion approach. This is illustrated in Fig. 5. Reductions in global warming are due to the lower GHG emissions in biobased glycerin production and to lower CO_2 emissions in fermentation and waste management subprocesses. Biobased glycerin increases bio-origin carbon in the media and the influent to the wastewater treatment facility. Including the alternative production system for biodiesel in the biobased glycerin product system produces more reduction in global warming in the system expansion approach than in the mass allocation method. The scenario analysis shows that using biobased glycerin as a substrate instead of petroleum-based glycerin can reduce GHG emissions of the immobilized enzyme production systems.

4 Conclusions, recommendations, and perspectives

The environmental life cycle inventories and impacts of the immobilized enzymes A, B, and C were estimated using modular techniques. Some of the general conclusions obtained from this LCA are:

- Enzyme A generally has larger environmental impacts than other enzymes because of (1) larger energy intensity and (2) lower enzyme production yield.
- The production of the immobilized enzymes analyzed is energy intensive, consuming about 117 to 207 MJ of nonrenewable energy per kilogram of immobilized enzyme.
- The global warming potential ranges from 16 to 25 kg CO_2 eq/kg immobilized enzyme.
- Immobilization and media preparation subprocesses are the primary local environmental impact sources (i.e., acidification, eutrophication, and photochemical smog formation).

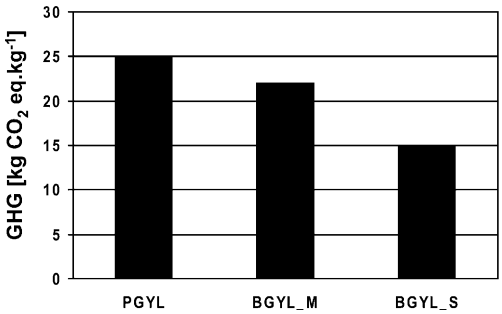

Fig. 5 Effects of biobased glycerin on enzyme A [*PGYL* petroleum-based glycerin, *BGYL_M* biobased glycerin with LCIs estimated from mass allocation, *BGYL_S* biobased glycerin with LCIs estimated from system expansion]

- The results of the Monte Carlo analysis suggest that the enzyme support, Sepabead, is one of the most environmentally sensitive factors in the immobilized enzymes
- The "what if" analysis shows that using biobased glycerin as a substrate instead of petroleum-based glycerin can reduce green house gas emissions of the immobilized enzyme production systems.
- The ranges of the environmental impacts estimated in this study are comparable to the environmental life cycle impacts for enzymes previously reported in the literature. Enzyme A is the only enzyme that exhibits larger impacts, presumably for the reasons discussed above.

Apparently, this modular methodology is satisfactory for the estimation of life cycle inventories and assessments for enzymes. This type of estimation therefore seems to be suitable for future life cycle assessments of biocatalyzed processes, especially when commercial LCI data on the enzymes are not readily available, as is often the case for enzymes produced in-house.

There is an ongoing need to obtain sufficiently accurate life cycle inventory data for substrates and biocatalysts in order to develop more accurate, transparent, and quantitative evaluations of the life cycle impacts of these bioprocesses. Future work will include integrating the results of this life cycle assessment into GlaxoSmithKline's FLASC™ database to be used as inputs for future life cycle assessments of biocatalyzed processes, to integrate human toxicity and ecotoxicity considerations, and to enhance the available data set on biobased materials. This would allow better life cycle estimations to be integrated into the development process at an earlier point in time, so that more sustainable processes and products may be designed.

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